Supporting Information

Synthesis of High Contrast Fluorescein-Diethers for Rapid Bench-Top Sensing of Palladium

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S1.1
Synthetic Methods

General Chemical Synthesis Methods. Compounds 3a-c were synthesized as previously described.1 All other reagents were the highest grade available and obtained from Sigma-Aldrich, Alfa Aesar, VWR International, or Fisher Scientific. Reactions were stirred under N₂(g) unless otherwise noted. Anhydrous solvents were drawn from Aldrich Sure-Seal bottles. Thin-layer chromatography was performed by using aluminum-backed plates coated with silica gel containing F₂₅₄ phosphor and visualized by UV-illumination or charring. Flash chromatography was performed on an Isolera system with SNAP columns (Biotage). The term “concentrated in vacuo” refers to the removal of solvents and other volatile materials by using a Büchi Rotavapor at variable pressure (controlled diaphragm pump; ≥1 mm Hg) while maintaining the water bath temperature below 40 °C. Mass spectrometry was performed by the Mass Spectrometry Center in the Department of Medicinal Chemistry at the University of Washington. NMR spectra were obtained with a JEOL ECX 400 MHz spectrometer at Wabash College. ¹H and ¹³C NMR spectra were referenced to residual solvent peaks.

Reduced fluorescein dimethoxybenzyl ester (4a). To a solution of fluorescein diacetate (3a, 153 mg, 0.367 mmol) in 15 mL EtOAc was added 10% w/w palladium on carbon (15 mg). The reaction flask was flushed thoroughly with nitrogen gas, followed by hydrogen gas. The reaction was stirred for 18 h at room temperature under 1 atm of hydrogen gas (balloon), after which time, the reaction flask was thoroughly purged with nitrogen gas. The crude reaction mixture was filtered through celite, rinsed with EtOAc (40 mL), and concentrated in vacuo, affording a white solid.

To a solution of the crude reduced fluorescein derivative in 6 mL CH₂Cl₂ was added N,N'-dicyclohexylcarbodiimide (DCC, 163.5 mg, 0.793 mmol, 2.2 eq), 4-dimethylaminopyridine (DMAP, 0.9 mg, 7.2 μmol, 0.02 eq), and 2,4-dimethoxybenzyl alcohol (DMB-OH, 133.3 mg, 0.793 mmol, 2.2 eq). The reaction was stirred at room temperature for 18 h. The mixture was deposited onto celite and purified by column chromatography (hexanes→2:3 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH₂Cl₂ additive), affording 207.3 mg of pure ester 4a as a white foamy solid (99% yield, 2 steps).

Data for 4a: Rf = 0.60 (3:1:1 v/v/v hexanes/EtOAc/CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃, δ): 2.29 (s, 6H, -COCH₃), 3.72 (s, 3H, -OCH₃), 3.82 (s, 3H, -OCH₃), 5.40 (s, 2H, -OCH₂Ar), 6.29 (s, 1H, Ar-H), 6.43 (d, J = 2.3 Hz, 1H, Ar-H), 6.47 (dd, J = 8.3, 2.4 Hz, 1H, Ar-H), 6.64 (dd, J = 8.4, 2.4 Hz, 2H, Ar-H), 6.98 (dd, J = 8.4, 0.7 Hz, 2H, Ar-H), 7.06 (dd, J = 7.9, 0.9 Hz, 1H, Ar-H), 7.19 (td, J = 7.8, 1.3 Hz, 1H, Ar-H), 7.30 (td, J = 7.6, 1.5 Hz, 1H, Ar-H), 7.34 (d, J = 8.3 Hz, Ar-H), 7.78 (dd, J = 7.8, 1.2 Hz, 1H, Ar-H); ¹³C NMR (100 MHz, CDCl₃, δ): 21.1, 24.7, 25.4, 34.9, 37.9, 55.3, 55.4, 55.8, 63.0, 98.4, 104.0, 109.7, 116.2, 116.7, 122.1, 126.4, 129.4, 130.2, 130.7, 131.7, 131.8, 132.4, 146.6, 149.8, 150.9, 159.2, 161.6, 168.5, 169.2.
Bis(allyl) leuco-fluorescein (6a). To a solution of triester 4a (207.3 mg, 0.365 mmol) in 8 mL THF and 4 mL MeOH was added ammonium bicarbonate (288.2 mg, 3.65 mmol, 10 eq) as a solution in 4 mL H2O. The reaction was stirred vigorously (~600 RPM) at room temperature for 60 h while shielded from light. The organic solvents were concentrated in vacuo and the pH was adjusted to ~3 with 10% w/v aqueous citric acid. The aqueous layer was diluted to 60 mL with H2O and extracted with EtOAc (3x60 mL). The organic layers were combined, dried over anhydrous MgSO4(s), filtered, and concentrated in vacuo. Purification by column chromatography (1:4 v/v EtOAc/hexanes→3:1 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH2Cl2 additive), afforded a white solid that turns yellow upon prolonged exposure to air with Rf = 0.20 (6:2:2 v/v/v hexanes/EtOAc/CH2Cl2).

To a solution of the diphenol intermediate in 8 mL CH2Cl2 and 8 mL H2O was added tetrabutylammonium hydrogensulfate (243.6 mg, 0.717 mmol, 2 eq), potassium carbonate (297.5 mg, 2.15 mmol, 6 eq), and allyl bromide (5, 186 µL, 2.15 mmol, 6 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The aqueous layer was diluted to 40 mL H2O and was extracted with CH2Cl2 (3x60 mL). The organic layers were combined, dried over anhydrous MgSO4(s), filtered, and concentrated in vacuo. Purification by column chromatography (hexanes→3:2 v/v hexanes/EtOAc, linear gradient) afforded pure diether 6a as a white foamy solid (126.1 mg, 62% yield over 2 steps).

Data for 6a: Rf = 0.24 (4:1 v/v hexanes/acetone); 1H NMR (400 MHz, CDCl3, δ): 3.72 (s, 3H, -OCH3), 3.81 (s, 3H, -OCH3), 4.51 (dt, J = 5.3, 1.5 Hz, 4H, -OCH2CHCH2), 5.29 (dq, J = 10.5, 1.4 Hz, 2H, -OCH2CHCH2), 5.40 (s, 2H, -OCH2CHCH2), 5.42 (m, 2H, -OCH2CHCH2) 6.05 (dd, J = 17.3, 10.5, 5.3 Hz, 2H, -OCH2CHCH2), 6.15 (s, 1H, Ar3CH), 6.48 (m, 4H, Ar-H), 6.64 (d, J = 2.6 Hz, 2H, Ar-H), 6.89 (d, J = 8.6 Hz, 2H, Ar-H), 7.04 (dd, J = 7.8, 1.1 Hz, 1H, Ar-H), 7.15 (td, J = 7.8, 1.1 Hz, 1H, Ar-H), 7.27 (m, 1H, Ar-H), 7.35 (d, J = 8.3, 1H, Ar-H), 7.37 (dd, J = 7.8, 1.4, 1H, Ar-H); 13C NMR (100 MHz, CDCl3, δ): 55.3, 55.4, 63.0, 68.9, 98.4, 101.8, 103.9, 110.8, 116.4, 117.2, 117.8, 125.9, 129.2, 130.1, 130.7, 131.4, 131.8, 132.1, 133.1, 147.9, 151.3, 158.1, 159.2, 161.5, 168.8.

Bis(allyl ether) fluorescein (7a). To a solution of diether 6a (126.1 mg, 0.223 mmol) in 9 mL CH2Cl2 and 1 mL H2O was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 152.1 mg, 0.670 mmol, 3 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The resulting mixture was filtered through a pad of celite and concentrated in vacuo. Purification by column chromatography (hexanes→7:3 v/v hexanes/acetone, linear gradient) afforded pure fluorescein diether 7a as a white foamy solid (84.1 mg, 91% yield).
Data for 7a:  
\[ R_f = 0.19 \text{ (4:1 v/v hexanes/acetone); } \]

**\(^1\)H NMR** (400 MHz, CDCl\(_3\), \(\delta\)): 4.56 (dt, \(J = 5.3, 1.5\) Hz, 4H, -OCH\(_2\)CH\(_2\)CH\(_2\)), 5.31 (dq, \(J = 10.5, 1.4\) Hz, 2H, -OCH\(_2\)C(CH\(_3\))\(_2\)), 5.43 (dq, \(J = 17.3, 1.5\) Hz, 2H, -OCH\(_2\)CHCH\(_2\)), 6.04 (ddt, \(J = 17.3, 10.5, 5.3\) Hz, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)), 6.63 (ABX, \(J_{AB} = 8.8\) Hz, 2H, Ar-H), 6.69 (ABX, \(J_{AB} = 8.8\) Hz, \(J_{AX} = 0\) Hz, 2H, Ar-H), 6.73 (dd, \(J = 17.3, 10.5, 5.3\) Hz, 2H, Ar-H), 6.80 (d, \(J = 2.4\) Hz, 1H, Ar-H), 7.00 (td, \(J = 7.5, 0.9\) Hz, 1H, Ar-H), 7.07 (td, \(J = 7.4, 1.3\) Hz, 1H, Ar-H), 7.08 (dd, \(J = 7.4, 1.0\) Hz, 1H, Ar-H);  
Collected HRMS (m/z): [M+H]\(^+\) calculated, 413.1389; observed, 413.1390.

**Reduced 2',7'-difluorofluorescein dimethoxybenzyl ester (4b)**. To a solution of 2',7'-difluorofluorescein diacetate (3b, 106.3 mg, 0.235 mmol) in 10 mL EtOAc was added 10% w/w palladium on carbon (10.6 mg). The reaction flask was flushed thoroughly with nitrogen gas, followed by hydrogen gas. The reaction was stirred for 18 h at room temperature under 1 atm of hydrogen gas (balloon), after which time, the reaction flask was thoroughly purged with nitrogen gas. The crude reaction mixture was filtered through celite, rinsed with EtOAc (30 mL), and concentrated in vacuo, affording a crude white solid.

To a solution of the crude reduced fluorescein derivative in 4 mL CH\(_2\)Cl\(_2\) was added N,N'-dicyclohexylcarbodiimide (DCC, 106.7 mg, 0.517 mmol, 2.2 eq), 4-dimethylaminopyridine (DMAP, 0.6 mg, 4.7 \(\mu\)mol, 0.02 eq), and 2,4-dimethoxybenzyl alcohol (DMB-OH, 87.0 mg, 0.517 mmol, 2.2 eq). The reaction was stirred at room temperature for 18 h. The mixture was deposited onto celite and purified by column chromatography (1:19 v/v EtOAc/hexanes \(\rightarrow\) 2:3 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH\(_2\)Cl\(_2\) additive), affording 125.4 mg of pure ester 4b as a white foamy solid (88% yield, 2 steps).

Data for 4b:  
\[ R_f = 0.56 \text{ (3:1:1 v/v/v hexanes/EtOAc/CH}_2\text{Cl}_2\text{); } \]

**\(^1\)H NMR** (400 MHz, CDCl\(_3\), \(\delta\)): 2.33 (s, 6H, -COC\(_3\)H), 3.76 (s, 3H, -OC\(_3\)H), 3.82 (s, 3H, -OC\(_3\)H), 5.42 (s, 2H, -OC\(_2\)Ar), 6.26 (s, 1H, Ar\(_3\)CH), 6.45 (d, \(J = 2.3\) Hz, 1H, Ar-H), 6.49 (dd, \(J = 8.2, 2.4\) Hz, 1H, Ar-H), 6.80 (d, \(J_{HF} = 10.4\) Hz, 2H, Ar-H), 6.90 (d, \(J_{HF} = 6.5\) Hz, 2H, Ar-H), 7.06 (dd, \(J = 7.9, 0.9\) Hz, 1H, Ar-H), 7.25 (td, \(J = 7.6, 1.2\) Hz, 1H, Ar-H), 7.35 (m, 2H, Ar-H), 7.83 (dd, \(J = 7.8, 1.4\) Hz, 1H, Ar-H);  
Collected HRMS (m/z): [M+Na]\(^+\) calculated, 627.1443; observed, 627.1427.
Bis(allyl) leuco-2',7'-difluorofluorescein (6b). To a solution of triester 4b (79.2 mg, 0.131 mmol) in 4 mL THF and 2 mL MeOH was added ammonium bicarbonate (103.5 mg, 1.31 mmol, 10 eq) as a solution in 2 mL H₂O. The reaction was stirred vigorously (~600 RPM) at room temperature for 18 h while shielded from light. The organic solvents were concentrated in vacuo and the pH was adjusted to ~3 with 10% w/v aqueous citric acid. The aqueous layer was diluted to 20 mL with H₂O and extracted with EtOAc (3x20 mL). The organic layers were combined, dried over anhydrous MgSO₄(s), filtered, and concentrated in vacuo. Purification by column chromatography (1:4 v/v EtOAc/hexanes→13:7 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH₂Cl₂ additive), afforded a white solid that turns yellow upon prolonged exposure to air with Rᶠ = 0.18 (3:1:1 v/v/v hexanes/EtOAc/CH₂Cl₂).

To a solution of the diphenol intermediate in 3 mL CH₂Cl₂ and 3 mL H₂O was added tetrabutylammonium hydrogensulfate (75.9 mg, 0.224 mmol, 2 eq), potassium carbonate (92.7 mg, 0.671 mmol, 6 eq), and allyl bromide (5, 58 µL, 0.67 mmol, 6 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The aqueous layer was diluted to 10 mL H₂O and was extracted with CH₂Cl₂ (3x20 mL). The organic layers were combined, dried over anhydrous MgSO₄(s), filtered, and concentrated in vacuo. Purification by column chromatography (hexanes→1:1 v/v hexanes/EtOAc, linear gradient) afforded pure diether 6b as a white foamy solid (50.9 mg, 65% yield over two steps).

Data for 6b: Rᶠ = 0.37 (4:1 v/v hexanes/EtOAc); ¹H NMR (400 MHz, CDCl₃, δ): 3.77 (s, 3H, -OCH₃), 3.82 (s, 3H, -OCH₃), 4.59 (dt, J = 5.4, 1.4 Hz, 4H, -OCH₂CHCH₂), 5.33 (dq, J = 10.5, 1.4 Hz, 2H, -OCH₂CHCH₂), 5.42 (s, 2H, -OCH₂Ar), 5.45 (dq, J = 17.3, 1.5 Hz, 2H, -OCH₂CHCH₂), 6.07 (ddt, J = 17.3, 10.5, 5.3 Hz, 2H, -OCH₂CCH₂), 6.12 (s, 1H, Ar₃H), 6.45 (d, J = 2.3 Hz, 1H, Ar-⁴H), 6.49 (dd, J = 8.3, 2.3 Hz, 1H, Ar-H), 6.68 (d, J_HF = 7.3 Hz, 2H, Ar-H), 6.72 (d, J_HF = 11.5 Hz, 2H, Ar-H), 7.03 (dd, J = 7.9, 1.0 Hz, 1H, Ar-H), 7.20 (td, J = 7.5, 1.3 Hz, 1H, Ar-H), 7.31 (td, J = 7.6, 1.4 Hz, 1H, Ar-H), 7.37 (d, J = 8.3 Hz, 1H, Ar-H), 7.79 (dd, J = 7.8, 1.4 Hz, 1H, Ar-H); ¹³C NMR (100 MHz, CDCl₃, δ): 55.3 (CH₃), 55.4 (CH₃), 63.1 (CH₂), 70.1 (CH₂), 98.5 (CH), 103.0 (CH), 103.0 (CH), 104.0 (CH), 115.9 (d, J_CF = 6.4 Hz, C), 116.2 (C), 116.3 (d, J_CF = 20.5 Hz, CH), 118.4 (CH₂), 126.4 (CH), 129.6 (CH), 129.9 (C), 131.2 (CH), 131.8 (CH), 132.3 (CH), 132.5 (CH), 146.0 (d, J_CF = 12.4 Hz, C), 146.4 (d, J_CF = 2.3 Hz, C), 146.7 (C), 148.7 (d, J_CF = 242 Hz, C), 159.2 (C), 161.6 (C), 168.3 (C).

Bis(allyl ether) 2',7'-difluorofluorescein (7b). To a solution of diether 6b (46.2 mg, 0.0773 mmol) in 4.5 mL CH₂Cl₂ and 0.5 mL H₂O was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 52.6 mg, 0.232 mmol, 3 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The resulting mixture was filtered...
through a pad of celite and concentrated in vacuo. Purification by column chromatography (hexanes→7:3 v/v hexanes/acetone, linear gradient) afforded pure 2',7'-difluorofluorescein diether 7b as a white foamy solid (32.9 mg, 95% yield).

Data for 7b: \( R_f = 0.20 \) (4:1 v/v hexanes/acetone); \(^1\)H NMR (400 MHz, CDCl\(_3\), \( \delta \)): 4.65 (d, \( J = 5.3 \) Hz, 4H, -OCH\(_2\)CHCH\(_2\)), 5.36 (dq, \( J = 10.5, 1.3 \) Hz, 2H, -OCH\(_2\)CHCH\(_2\)), 5.47 (dq, \( J = 17.3, 1.4 \) Hz, 2H, -OCH\(_2\)CHCH\(_2\)), 6.06 (ddt, \( J = 17.3, 10.5, 5.3 \) Hz, 2H, Ar-\( \text{H} \)), 6.45 (d, \( J_{HC} = 11.1 \) Hz, 2H, Ar-\( \text{H} \)), 6.82 (d, \( J_{HC} = 7.1 \) Hz, 2H, Ar-\( \text{H} \)), 7.02 (ddt, \( J = 17.3, 10.5, 5.3 \) Hz, 2H, Ar-\( \text{H} \)), 7.21 (dd, \( J = 7.1, 0.9 \) Hz, 1H, Ar-\( \text{H} \)), 7.35 (s, 1H, Ar-\( \text{H} \)), 7.47 (s, 1H, Ar-\( \text{H} \)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\), \( \delta \)): 70.1 (CH\(_2\)), 82.4 (C), 102.8 (d, \( J_{CF} = 1.6 \) Hz, CH), 109.7 (d, \( J_{CF} = 6.1 \) Hz, C), 113.9 (d, \( J_{CF} = 21.0 \) Hz, CH), 118.8 (CH\(_2\)), 123.8 (CH), 125.3 (CH), 126.5 (C), 130.2 (CH), 131.9 (CH), 135.3 (CH), 147.8 (d, \( J_{CF} = 2.3 \) Hz, C), 148.7 (d, \( J_{CF} = 12.6 \) Hz, C), 151.1 (d, \( J_{CF} = 190 \) Hz, C), 168.7 (C); HRMS (m/z): [M+H]\(^+\) calculated, 449.1201; observed, 449.1212.

Reduced 2',7'-dichlorofluorescein dimethoxybenzyl ester (4c). To a solution of 2',7'-dichlorofluorescein diacetate (3c, 150. mg, 0.309 mmol) in 8 mL EtOAc was added 10% w/w palladium on carbon (15 mg). The reaction flask was flushed thoroughly with nitrogen gas, followed by hydrogen gas. The reaction was stirred for 18 h at room temperature under 1 atm of hydrogen gas (balloon), after which time, the reaction flask was thoroughly purged with nitrogen gas. The crude reaction mixture was filtered through celite, rinsed with EtOAc (30 mL), and concentrated in vacuo, affording a crude white solid.

To a solution of the crude reduced fluorescein derivative in 6 mL CH\(_2\)Cl\(_2\) was added \( N,N' \)-dicyclohexylcarbodiimide (DCC, 140.3 mg, 0.680 mmol, 2.2 eq), 4-dimethylaminopyridine (DMAP, 0.8 mg, 6.2 \( \mu \)mol, 0.02 eq), and 2,4-dimethoxybenzyl alcohol (DMB-OH, 114.4 mg, 0.680 mmol, 2.2 eq). The reaction was stirred at room temperature for 18 h. The mixture was deposited onto celite and purified by column chromatography (hexanes→2:3 v/v CH\(_2\)Cl\(_2\)/hexanes, linear gradient, with constant 20% v/v CH\(_2\)Cl\(_2\) additive), affording 149.4 mg of pure ester 4c as a white foamy solid (76% yield, 2 steps).

Data for 4c: \( R_f = 0.67 \) (3:1 v/v hexanes/EtOAc/CH\(_2\)Cl\(_2\)); \(^1\)H NMR (400 MHz, CDCl\(_3\), \( \delta \)): 2.34 (s, 6H, -COCH\(_3\)), 3.74 (s, 3H, -OCH\(_3\)), 3.82 (s, 3H, -OCH\(_3\)), 5.41 (s, 2H, -OCH\(_3\)Ar), 6.27 (s, 1H, Ar-\( \text{H} \)), 6.44 (d, \( J = 2.1 \) Hz, Ar-\( \text{H} \)), 6.49 (dd, \( J = 8.3, 2.4 \) Hz, 1H, Ar-\( \text{H} \)), 6.93 (s, 2H, Ar-\( \text{H} \)), 7.05 (m, 3H, Ar-\( \text{H} \)), 7.27 (m, 1H, Ar-\( \text{H} \)), 7.37 (m, 2H, Ar-\( \text{H} \)), 7.85 (dd, \( J = 7.8, 1.2 \) Hz, 1H, Ar-\( \text{H} \)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\), \( \delta \)): 20.6 (CH\(_3\)), 55.4 (CH\(_3\)), 63.2 (CH\(_2\)), 98.5 (CH), 104.2 (CH), 111.9 (CH), 116.0 (C), 121.3 (C), 123.1 (C), 127.0 (CH), 130.0 (C), 130.1 (C), 130.7 (CH), 131.6 (C), 131.8 (CH), 132.8 (CH), 146.1 (C), 149.2 (C), 159.1 (C), 161.6 (CH), 168.1 (C), 168.2 (C).
Bis(allyl) leuco-2',7'-dichlorofluorescein (6c). To a solution of triester 4c (149.4 mg, 0.234 mmol) in 8 mL THF and 4 mL MeOH was added ammonium bicarbonate (185.2 mg, 2.34 mmol, 10 eq) as a solution in 4 mL H₂O. The reaction was stirred vigorously (~600 RPM) at room temperature for 18 h while shielded from light. The organic solvents were concentrated in vacuo and the pH was adjusted to ~3 with 10% w/v aqueous citric acid. The aqueous layer was diluted to 30 mL with H₂O and extracted with EtOAc (3x30 mL). The organic layers were combined, dried over anhydrous MgSO₄, filtered, and concentrated in vacuo. Purification by column chromatography (1:4 v/v EtOAc/hexanes→3:1 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH₂Cl₂ additive), afforded a white solid that turns yellow upon prolonged exposure to air with Rᵥ = 0.23 (3:1:1 v/v/v hexanes/EtOAc/CH₂Cl₂).

To a solution of the diphenol intermediate in 6 mL CH₂Cl₂ and 6 mL H₂O was added tetrabutylammonium hydrogensulfate (159.2 mg, 0.469 mmol, 2 eq), potassium carbonate (194.3 mg, 1.41 mmol, 6 eq), and allyl bromide (5, 122 µL, 1.41 mmol, 6 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The aqueous layer was diluted to 20 mL H₂O and was extracted with CH₂Cl₂ (3x30 mL). The organic layers were combined, dried over anhydrous MgSO₄(s), filtered, and concentrated in vacuo. Purification by column chromatography (hexanes→2:3 v/v EtOAc/hexanes, linear gradient) afforded pure diether 6c as a white foamy solid (121.6 mg, 79% yield over two steps).

Data for 6c: Rᵥ = 0.68 (3:1:1 v/v/v hexanes/EtOAc/CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃, δ): 3.76 (s, 3H, -OC₃H₃), 3.82 (s, 3H, -OC₃H₃), 4.61 (d, J = 4.9 Hz, 4H, -OCH₂CHCH₂), 5.34 (dq, J = 10.6, 1.4 Hz, 2H, -OCH₂CHCHHH), 5.42 (s, 2H, -OCH₂Ar), 5.49 (dq, J = 17.3, 1.5 Hz, 2H, -OCH₂CHCHH), 6.07 (ddt, J = 17.3, 10.6, 5.2 Hz, 2H, -OCH₂CHCH₂), 6.14 (s, 1H, Ar-CH), 6.44 (d, J = 2.4 Hz, 1H, Ar-H), 6.50 (dd, J = 8.3, 2.4 Hz, 1H, Ar-H), 6.65 (s, 2H, Ar-H), 7.00 (m, 3H, Ar-H), 7.20 (td, J = 7.6, 1.3 Hz, 1H, Ar-H), 7.30 (td, J = 7.6, 1.5 Hz, 1H, Ar-H), 7.37 (d, J = 8.3 Hz, 1H, Ar-H), 7.81 (dd, J = 7.8, 1.2 Hz, 1H, Ar-H); ¹³C NMR (100 MHz, CDCl₃, δ): 55.4 (CH₃), 55.4 (CH₃), 63.1 (CH₂), 69.8 (CH₂), 98.6 (CH), 101.7 (CH), 104.0 (CH), 116.2 (C), 117.3 (C), 117.4 (C), 118.1 (CH₂), 126.5 (CH), 129.8 (CH), 129.9 (C), 130.7 (CH), 131.3 (CH), 131.8 (CH), 132.3 (CH), 132.4 (CH), 149.5 (C), 153.3 (C), 159.1 (C), 161.6 (C), 168.2 (C).

Bis(allyl ether) 2',7'-dichlorofluorescein (7c). To a solution of diether 6c (121.6 mg, 0.192 mmol) in 9 mL CH₂Cl₂ and 1 mL H₂O was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 130.7 mg, 0.576 mmol, 3 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The resulting mixture was filtered through a pad
of celite and concentrated in vacuo. Purification by column chromatography (hexanes→7:3 v/v hexanes/acetone, linear gradient) afforded pure 2’,7’-dichlorofluorescein diether 7c as a white foamy solid (77.1 mg, 83% yield).

Data for 7c: \( R_f = 0.19 \) (4:1 v/v hexanes/acetone); \(^1\)H NMR (400 MHz, CDCl\(_3\), \( \delta \)): 4.65 (d, \( J = 4.9 \) Hz, 4H, -OCH\(_2\)CH\(_2\)H), 5.36 (dq, \( J = 10.6, 1.4 \) Hz, 2H, -OCH\(_2\)CH\(_2\)H), 5.50 (dq, \( J = 17.3, 1.5 \) Hz, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)H), 6.06 (ddt, \( J = 17.3, 10.6, 5.2 \) Hz, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)H), 6.74 (s, 2H, Ar-H), 6.77 (s, 2H, Ar-H), 7.16 (d, \( J = 7.5 \) Hz, 1H, Ar-H), 7.68 (td, \( J = 7.5, 1.2 \) Hz, 1H, Ar-H), 7.72 (td, \( J = 7.3, 1.3 \) Hz, 1H, Ar-H), 8.06 (d, \( J = 7.1 \) Hz, 1H, Ar-H); \(^13\)C NMR (100 MHz, CDCl\(_3\), \( \delta \)): 69.8 (CH\(_2\)), 81.8 (C), 101.5 (CH), 111.4 (C), 118.5 (CH), 118.7 (C), 123.8 (CH), 125.4 (CH), 126.4 (C), 128.8 (CH), 130.3 (CH), 131.7 (CH), 135.4 (CH), 150.5 (C), 152.0 (C), 155.5 (C), 168.8 (C); HRMS (m/z): [M+H]\(^+\) calculated, 481.0610; observed, 481.060.
Optical Methods

General Optical Spectroscopy Methods. Fluorescence emission was measured in a 1-cm path length, 3.5 mL glass cuvette from Starna Cells in ACS spectrophotometric grade methanol at ambient temperature (22 ± 2 °C) and recorded on a Carey Eclipse Fluorescence Spectrometer (Varian). Samples containing compounds 7a and 7b were excited at 496 nm and the emission intensities were collected at 525 nm. The excitation and emission slits were set at 2.5 nm and the PMT Detector Voltage was 550 V. Samples containing compound 7c were excited at 504 nm and the emission intensities were collected at 529 nm. The excitation and emission slits were set at 2.5 nm and the PMT Detector Voltage was 550 V.

Preparation of stock solutions. 10.0 mM stock solution of compounds 7a-c were prepared in DMSO and stored in a freezer when not in use. A 100. mM solution of tri(2-furyl) phosphine (TFP) in DMSO was freshly prepared before each experiment. A solution of NaBH₄ (2.5 M in 10 N NaOH) was stored at ambient temperature, and a 100 mM solution of NaBH₄ was freshly prepared before each experiment by diluting the 2.5 M solution with pH 9 borate buffer. A 10.0 μM solution of Pd from Pd₂(allyl)₂Cl₂ in MeOH was freshly prepared before each experiment, from which serial dilutions provided a variety of palladium concentrations for testing.

Kinetic Data for Palladium Sensors 7a-c. A solution of TFP (10 μL, 100 mM in DMSO), NaBH₄ (25 μL, 100 mM in pH 9 borate buffer), and the palladium sensor being tested (2.5 μL, 10 mM in DMSO) was added to 1.94 mL of ACS spectrophotometric grade methanol. Varying concentrations of palladium were added (20 μL, 10 μM→13.7 nM in MeOH) and the sample was stirred while fluorescence emission was recorded every 0.2 min.

Figure S1. Representative Kinetic Data for fluorescein bis(allyl ether) 7a. Inset is a zoomed in region for the lowest concentrations. Final rate of fluorescence increase used for Figure 2 was based on four trials.
Figure S2. Representative Kinetic Data for 2’,7’-difluorofluorescein bis(allyl ether) 7b. Inset is a zoomed in region for the lowest concentrations. Final rate of fluorescence increase used for Figure 2 was based on five trials.

Figure S3. Representative Kinetic Data for 2’,7’-dichlorofluorescein bis(allyl ether) 7c. Inset is a zoomed in region for the lowest concentrations. Final rate of fluorescence increase used for Figure 2 was based on five trials.
Figure S4. Comparison of fluorogenic bis(allyl ether) fluorescein derivatives 7a-c as shown in Figure 2, with an inset to compare rates in the ppt range. The slope is calculated from the linear portion of the kinetic trace resulting from the addition of varying concentrations of palladium to a solution of 125 μM of the probe, 500 μM TFP, and 1.25 mM NaBH₄ in MeOH. This data is derived from several trials as shown in Figure S1-S3.

Figure S5. Direct comparison between the assay described by Koide et al with sensor 1 (i and ii) and the reported sensor 7c (iii and iv) after 1 h stirring, as called for in Koide’s protocol. The vials contain: (i) no palladium with 1, (ii) 1 ppb palladium with 1, (iii) no palladium with 7c, and (iv) 1 ppb palladium with 7c. This photo shows a moderate increase in fluorescence of 1 with 1 ppb palladium (i and ii) after an hour of stirring at 45°C, as reported previously, but background fluorescence would make visual detection of lower concentrations inconclusive. Importantly, after 1 h, the sample containing 7c without palladium added (iii) is still nonfluorescent due to the stability of the ether functionality.

Limit of Detection. The limits of detection for sensors 7a-c were determined from plots of the average fluorescence intensity at a single time point in the kinetic traces vs. the concentration of palladium(0). From the linear regression (Microsoft Excel) analysis of the resulting plots, the
limits of detection (LOD) were calculated using the following equation:

\[ \text{LOD} = \frac{3 s_b}{m} \]

where \( m \) is the slope of the regression line and \( s_b \) is the standard deviation of the y-intercept. The limits of detection for these palladium sensors were found to be around 0.5 ppb (see Table S1). However, Figure 4 demonstrates that the presence of palladium(0) in concentrations as low as 67.5 ppt can be visually identified.

**Figure S6.** The average fluorescence measurement from kinetic traces for sensors 7a-c at various palladium concentrations and a single time point: (a) 7a at 5 min; (b) 7b at 2 min; (c) 7c at 2 min.

**Table S1.** Table of parameters used for determination of limit of detection.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Reaction time</th>
<th>Slope (m)</th>
<th>( s_b )</th>
<th>LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>5 min</td>
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<td>1.23</td>
<td>0.673 ppb</td>
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<tr>
<td>7b</td>
<td>2 min</td>
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<td>13.9</td>
<td>0.455 ppb</td>
</tr>
<tr>
<td>7c</td>
<td>2 min</td>
<td>121</td>
<td>19.7</td>
<td>0.488 ppb</td>
</tr>
</tbody>
</table>
